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Crop productivity and chemical compositions of black cumin essential oil in sole crop and intercropped with soybean under contrasting fertilization

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A R T I C L E I N F O

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A B S T R A C T

Intercropping systems and the application of organic manures play an important role in increasing of quantity and quality of plant products. In order to evaluate the quantity and quality of black cumin products under contrasting fertilization, a factorial experiment was conducted in a randomized complete block design with three replications in 2016. Soybean and black cumin seeds were sown in five planting patterns (sole black cumin, sole soybean and three intercropping ratios of soybean: black cumin (1:2): one row of soybean plus two rows of black cumin, soybean: black cumin (1:1): one row of soybean plus one row of black cumin and soybean: black cumin (2:1): two rows of soybean plus one row of black cumin. All these cropping systems were received organic manure and chemical fertilizer. The results indicated that the highest seed yield (on an average by 86 g m^{-2}) of black cumin was achieved in sole crop of black cumin treated with organic manure. The maximum seed yield of soybean (on an average by 247 g m^{-2}) and land equivalent ratio (1.06) was obtained in two rows of soybean + one row of black cumin under the application of chemical fertilizer. The p-cymene (20.51–62.77%), carvacrol (2.40–25.99%), longifolene (1.11–24.69%) and spathulenol (0.9–14.45%) were major chemical compositions of black cumin. The highest content of p-cymene and carvacrol of black cumin essential oil were recorded in one row of soybean + two rows of black cumin with the application of chemical fertilizer and one row of soybean + one row of black cumin under chemical fertilizer, respectively. The highest longifolene and spathulenol content was observed in one row of soybean + two rows of black cumin treated with organic manure. These major chemical compositions are useful for industrial use (food and pharmaceutical). Therefore, according to different subjects of applying in industries it could be suggested especial treatment with favorite major compounds.

1. Introduction

Intercropping system is considered as the simultaneous cultivation of two or more crops in the same field and is a more sustainable method for enhancing crop yields in comparison with sole cropping systems (Ehrmann and Ritz, 2014; Brooker et al., 2015). Complementarity in resource niches such as diff ;erent rooting depth, diff ;erential canopy architecture or diff ;erential resource use (as with nitrogen), are also other contributing factors to greater yield stability (Raseduzzaman and Jensen, 2017).

Previous studies have shown that legume plants are key species in increasing resource efficiency (Fallah et al., 2018). Adding legumes in intercropping systems is justified by their ability to fix atmospheric nitrogen (Duchene et al., 2017). Also, Intercropping system can provide many ecosystem services such as decreasing chemical inputs requirements for the purpose of control weeds (Letourneau et al., 2011; Iverson et al., 2014), insect pests (Liebman and Dyck, 1993) and plant diseases (Boudreau, 2013). One intercrop component may change the micro-climate for another component, which could be unfavorable for pest and disease attack, resulting in greater productivity and stability (Raseduzzaman and Jensen, 2017).

Previous studies have mainly focused on intercropping of legume with non-legume (Lithourgidis et al., 2011; Dabbagh Mohammadi Nassab et al., 2011; Franco et al., 2015). At present, intercropping of legume with medicinal plants is increasing. Intercropping of legume with medicinal plants, improve the quality and quantity of essential oil in medicinal and aromatic plants (Fallah et al., 2018; Amani Machiani et al., 2018a). Soybean is known to be a leguminous crop and an important source of food in many countries (Cabrera et al., 2015). Nitrogen fixation in soybean plant is the result of symbiosis between

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soybean roots and bacterium *Bradyrhizobium japonicum* (Fan et al., 2017). The amount of nitrogen fixation by soybean plant is estimated to be 65-115 kg ha^{-1} during the growing season (Amani Machiani et al., 2018a).

One of the most important medicinal plants is black cumin (*Nigella sativa* L.). This plant is an annual plant belonging to the family Ranunculaceae, which is cultivated in the countries around Mediterranean Sea (Gharibzahedi et al., 2010).

The components of the essential oil are used in industry. P-cymene is commonly used in pastries and to flavor beverages (Sintim et al., 2015). Carvacrol has been shown to exhibit anti-mutagenic, anti-inflammatory, anti-microbial, anti-platelet, anti-oxidant, analgesic, anti-angiogenic, anti-parasitic, anti-elastase, insecticidal and cell-protective characteristics (Sokmen et al., 2004; Can Baser, 2008). Spathulenol is used as a base material in various fields such as medicines, foods, detergents, toothpaste and cosmetics (Ziaei et al., 2011; Paksoy et al., 2016). Longifolene is used in perfumery industry due to its woody odor (Tyagi et al., 2009).

Although chemical fertilizers are widely applied to increase crop yield, but their long-term application enhances soil pH, reduces beneficial soil microflora, pollutes water bodies and imbalances soil ecological system (Ahmadian et al., 2011; Bistgani et al., 2018). Consequently, in recent years, demand for organic products has increased due to health and sustainable environment consideration (Sangkumchaliang and Huang, 2012) particularly for medicinal products. On the other hand, a trend of medicinal plants cultivation with the application of organic manures is increasing at a rapid pace (Bajeli et al., 2016). Organic manure contains essential nutrients for crop growth. It improves chemical and physical properties of the soil and enhances organic matter, cation exchange and water holding capacity and ultimately the yield (Schlegel et al., 2015a,b; Pandey et al., 2016). Also it can effect on chemical compositions and the quality of medicinal plants (Fallah et al., 2018).

Previous studies have exposed that the application of organic manure has enhanced the quantity and quality of plant products. Abou El-Magd et al., (2008) reported the addition of organic manure enhanced the seed yield of fennel. Fallah et al. (2018) found that the application of broiler litter increases aerial yield, essential oil yield of dragonhead. Bajeli et al. (2016) reported that the use of organic manures (poultry manure, farmyard manure and vermicopmost) enhances aerial yield, oil yield and menthol content of the mint. Pandey et al. (2016) illustrated that the farmyard manure and poultry manure enhances methyl chavicol content of basil.

In the present study, the specific objectives were (a) to study the effects of chemical fertilizer and organic manure on black cumin yield in sole crop and intercrop, (b) to compare the essential oil production and chemical composition in black cumin plants cultivated under contrasting fertilization and (c) to assess the advantage of black cumin/soybean intercropping over sole cropping through the use of land equivalent ratio (LER).

2. Materials and methods

2.1. Experimental set up

Field experiment was carried out in 2016 growing season at research farm of Shahrekord University (50° 49´E, 32° 21´N; 2050 m above sea level). The experiment was carried out with a factorial arrangement based on randomized complete block design with three replications. Soybean and black cumin seeds were sown in five planting patterns (sole crop soybean (D), sole crop black cumin (S) and three intercropping ratios of soybean: black cumin (1:2): one row of soybean + two rows of black cumin, soybean: black cumin (1:1): one row of soybean + one row of black cumin and soybean: black cumin (2:1): two rows of soybeans + one row of black cumin)). All these treatments were applied with organic manure and chemical fertilizer.

The climate of the region is classified by a temperate and cold climate with warm and dry summer, with average annual precipitation of 316 mm and an average monthly temperature of 12.12 °C. For soil analysis, soil samples were randomly collected from a depth of 0–30 cm at ten points using a soil auger. All soil samples were air-dried in the laboratory for 4 days and then crushed and sieved through a 2 mm sieve. The physical and chemical properties of the soil and the broiler litter were analyzed. The research farm soil is Entisol. The others properties of soil are as follows: pH: 8.09, electrical conductivity (EC): 0.587 (dS m^{-1}), total nitrogen: 0.59 (g kg^{-1}), available phosphorus: 0.021 (g kg^{-1}), available potassium: 0.313 (g kg^{-1}). The characteristics of broiler litter are as follows: pH: 7.56, electrical conductivity (EC): 5.57 (dS m^{-1}), total nitrogen: 19.1 (g kg^{-1}), total phosphorus: 6.9 (g kg^{-1}), total potassium: 12.9 (g kg^{-1}).

In this experiment, the sources of chemical nitrogen and phosphorus were urea (46% N) and triple superphosphate (44% P_2O_5), respectively. Chemical fertilizers (152 kg ha^{-1} of urea and 141 kg ha^{-1} of triple superphosphate) and organic manure (7.4 Mg ha^{-1}) were added before sowing. The black cumin and soybean seeds were obtained from Pakan Bazr Company, Isfahan, Iran, and Lorestan Agriculture and Natural Resource Research Center, respectively. Soybean seeds were inoculated with commercial rhizobia and sown 15 min after inoculation on 23th May 2016 simultaneously with black cumin. The soybean and black cumin were sown at 30 and 80 seeds m^{-2} in both the sole and intercropped plots, respectively. The sole and intercropped soybean and black cumin were sown in the same rows spacing. The size of each plot was 2 × 5 m and consisted of 12 rows. The distance between rows spacing was 0.5 m. The crops were cultivated according to organic agriculture practices with no use of herbicide or pesticide applications. Weeds were controlled by hand weeding during the growing season.

2.2. Determination of soybean and black cumin seed yield

The black cumin and soybean were harvested at maturity stage (133 and 126 days after sowing), respectively. The plants were cut at ground level from each plot with manual shears. For yield determination of the soybean and black cumin, samples were transferred to the laboratory and kept at 70 °C to dry in an oven for 2 days. After drying, the seed yield was measured in g m^{-2}.

2.3. Nitrogen and phosphorus concentration

The nitrogen content of the black cumin and soybean seeds was determined using the KJeldahl method. The phosphorus content was determined spectrophotometerically with a Pharmacia LKB Novaspec-II spectrophotometer.

2.4. Isolation of essential oil

The seeds of the black cumin were harvested at the maturity stage (133 days after sowing), shade-dried and powderded. The amount of essential oil of black cumin seeds was determined through water and steam distillation and by means of Kaiser-Long Apparatus. The samples of essential oils were dehydrated over anhydrous sodium sulphate and stored at 4 °C until gas chromatography-mass spectrometry (GC–MS). The essential oil yield was calculated using the following equation (Bistgani et al., 2018):

Essential oil yield (g m^{-2}): (Black cumin seed yield g m^{-2} × essential oil content g kg)/1000

2.5. Gas chromatography

Gas chromatography (GC) was carried out using a Thermoquest-Finnigan device equipped with FID detector and DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed as follows: The oven temperature was initiated at

60 °C, then was enhanced from 60 to 250 °C at a rate of 5 °C/min and held for 10 min at 250 °C. The injection temperature was set at 250 °C. The injector was set in a split mode (split ratio of 1:100). The analyses were carried out using helium as the carrier gas at the flow rate of 1/1 ml/min.

2.6. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a Thermoquest-Finnigan device equipped with a DB-5 column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). Helium was used as the carrier gas (flow rate: 1.1 mL/min). The oven temperature was programmed as follows: The oven temperature was initiated at 60 °C, then increased from 60 to 250 °C at a rate of 5 °C /min and held for 10 min at 250 °C. The mass spectra were recorded at 70 eV of ionization voltage. Mass range acquisition was from 40 to 400 *m/z*. The ionization mode was electron impact (EI) and the temperature of the ionization source was kept at 200 and 250 °C.

2.7. Identification of essential oil chemical composition

The chemical composition of the samples was verified by comparing their mass spectra with those held in the computer library or achieved using authentic components. Retention indices were measured using the retention times of *n*-alkanes (C_6-C_{24}) injected after the essential oil under the same conditions. The identified components were confirmed by comparison with relative retention indices, either with those of authentic components or with data published in the literature (Adams, 2007; Harzallah et al., 2011).

2.8. Land equivalent ratio

The advantage of intercropping and the effect of competition between intercropping components were assessed by calculating the land equivalent ratio (LER) index. The LER calculated as (Mao et al., 2012):

$$LER = LER_s + LER_b \quad (1)$$

$$LER_s = Y_{si}/Y_s \quad (2)$$

$$LER_b = Y_{bi}/Y_b \quad (3)$$

where Y_b and $Y_s$are the yields of black cumin and soybean, respectively, as sole crops and Y_{bi} and Y_{si} are the yields of black cumin and soybean, respectively, as intercropps.

A LER value lower than 1.0 indicates mutual antagonism in the intercropping system; thus, a LER value of less than 1.0 has no intercropping advantage and reveals that interspecific competition is higher than interspecific facilitation in the intercropping system. A LER value of 1.0 indicates that the two intercropped species make similar demands on the same limited resources. A LER value greater than 1.0 indicates an intercropping advantage or demonstrates that the interspecific competition is lower than interspecific facilitation, meaning that intercropping resulted in greater land-use efficiency (Fetene, 2003; Wahla et al., 2009).

2.9. Statistical analysis

Analysis of variance (ANOVA) was performed as a factorial experiment in a randomized complete block design using SAS software (version 9; SAS Institute; USA). Each data point was the mean of three replications. The mean of the treatments was compared using the least significant difference (LSD) test at the 5% probability level. To evaluate likely similarities among the chemical compositions of the essential oils of the dragonhead from different cropping patterns and fertilization sources, hierarchical cluster analysis was performed using SPSS (ver. 16) based on the Ward method.

Table 1
Analysis of variance (*P* value) for effect of cropping system on yield and nitrogen concentration of soybean and black cumin under contrasting fertilization.

Source of variation	Seed yield		Nitrogen concentration	
	Soybean	Black cumin	Soybean	Black cumin
Replication	NS	0.05	NS	NS
Cropping system (C)	0.05	0.001	NS	0.05
Fertilization source (F) (dS/m)	0.05	0.001	NS	NS
C × F	0.01	0.01	NS	NS

NS represents non-significant.

3. Results and discussion

3.1. The yield of black cumin and soybean

According to the results, the effect cropping pattern ($P \le 0.05$), fertilization source ($P \le 0.05$) and the interaction of cropping pattern × fertilization source ($P \le 0.01$) on the yield of soybean were significant (Table 1). In the sole cropped soybeans, soybean: black cumin (1:2) and soybean: black cumin (1:1), the application of organic manure, enhanced the yield of soybean by 5, 41 and 50%, respectively, compared with chemical fertilizer; although, this increase was not considerable in the sole crop (Fig. 1). Adversely, in the pattern of soybean: black cumin (2:1), the addition of chemical fertilizer increased the yield of soybean by 14%; although, this increase was not significant. The highest seed yield of soybean (average of 247 g m^{-2}) was obtained in soybean: black cumin (2:1) treated with chemical fertilizer.

In soybean-black cumin intercropping system, the seed yield of soybean enhanced. The higher production of soybean in intercropping method, might be the result of its better ability to be the soil resource and to capture light or a the combination of both (Dabbagh Mohammadi Nassab et al., 2011). Soybean with bigger or wider leaves has better ability to capture light than the black cumin (Dabbagh Mohammadi Nassab et al., 2011). Our result were in agreement with those of Fallah et al. (2018) who noted that intercropping soybean with dragonhead would enhance the seed yield of soybean. Weisany et al. (2016) reported that intercropping common bean with dill increases the seed yield of common bean.

The application of organic manure did enhance soybean seed yield with the exception of soybean: black cumin (2:1). The decrease in soybean seed yield in this treatment can be possibly attributed to the higher supply of nitrogen. Nearly all legumes (such as soybean) fix less atmospheric nitrogen if the soil has high nitrogen content (Ghosh et al., 2004). It seems that higher availability of nitrogen in organic manure

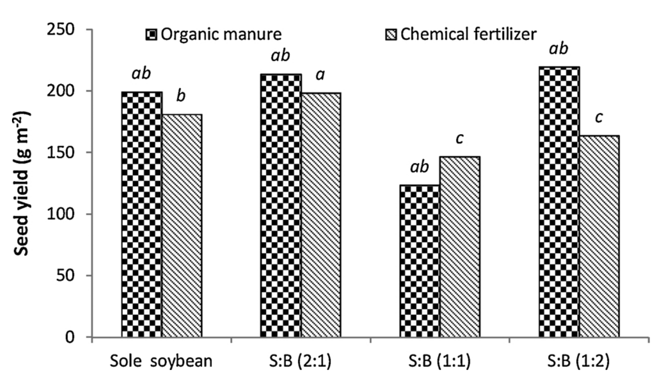

Fig. 1. The effect of cropping system on seed yield of soybean under contrasting fertilization. Means with different letters are significantly different, according to LSD test at $P < 0.05$. S and B are presented soybean and black cumin, respectively.

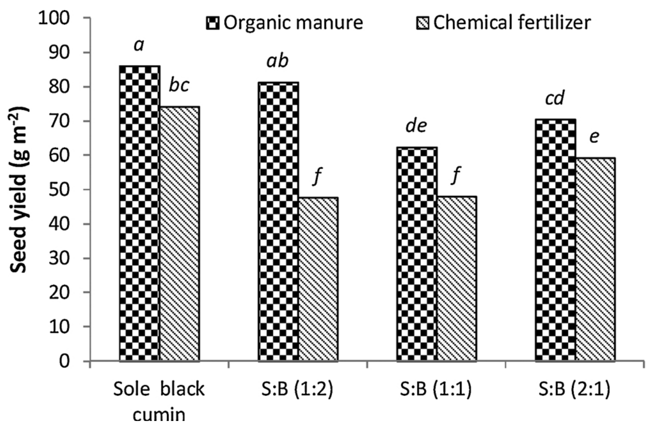

Fig. 2. The effect of cropping system on seed yield of black cumin under contrasting fertilization. Means with different letters are significantly different, according to LSD test at $P < 0.05$. S and B are presented soybean and black cumin, respectively.

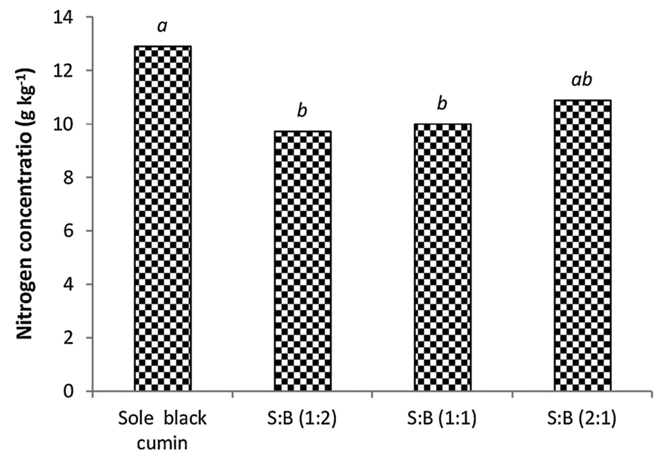

Fig. 3. The effect of cropping system on nitrogen concentration of black cumin. Means with different letters are significantly different, according to LSD test at $P < 0.05$. S and B are presented soybean and black cumin, respectively.

reduces nitrogen fixation and consequently the seed yield.

Variance analysis of the results indicated that the effect cropping pattern, fertilization source and cropping pattern × fertilization source interaction on the seed yield of black cumin were significant (Table 1). In the case of black cumin, the application of organic manure significantly enhanced seed yield in sole crop and also three patterns of intercropping. The greatest seed yield of black cumin was recorded in sole cropped plots under organic manure treatment (Fig. 2). The decrease in seed yield of black cumin can be passilbly attributed to lower light availability due to shading by relatively tall soybean (Nambiar et al., 1983). Also this shading effect decrease growth attributes such as leaf area, leaf area index and dry matter accumulation per plant (Jannoura et al., 2014). Amani Machiani et al. (2018a, b) found that intercropping soybean with peppermint and faba bean with peppermint would decrease peppermint yield in comparison with the sole crop. These researchers all reported that the highest peppermint yield in both harvest time (July and October) were obtained in sole crop system.

The application of organic manure enhanced the seed yield of black cumin in both cropping systems (sole crop and intercropping). Organic manures contain essential nutrients for crop growth. They improve chemical and physical properties of the soil and enhance organic matter, cation exchange and water holding capacity and ultimately the yield (Schlegel et al., 2015a, b; Pandey et al., 2016). Previous studies have revealed that the application of organic manure enhanced yield of medicinal plants. For example, Abou El-Magd et al., (2008) reported that the addition of organic manure enhances seed yield of fennel. Fallah et al. (2018) found that the application broiler litter increases the yield of dragonhead. Bajeli et al., (2016) also observed that the use of organic manure enhances yield of mint.

3.2. Nitrogen and phosphorus concentration

The ANOVA analysis showed that soybean nitrogen concentration is not affected by cropping pattern, fertilization source and their interactions (Table 1).

The result also indicated that black cumin nitrogen concentration is affected by cropping pattern ($P \le 0.05$); however, this trait is not affected by fertilization source and the interaction between cropping patterns and fertilization source (Table 1). The nitrogen concentration of black cumin was similar in both sole crop and soybean: black cumin (2:1) (Fig. 3). Also, nitrogen concentration was similar in three patterns of intercropping. The highest nitrogen concentration was recorded in the sole cropped plots. The increase in nitrogen concentration of sole cropped black cumin can be attributed to better utilization of nitrogen sources (soil and fertilizers) without the soybean. Inal et al. (2007) reported that intercropping of peanut (*Arachis hypogaea* L.) with maize decreased maize nitrogen concentration.

The effect of cropping pattern ($P \le 0.001$), fertilization source ($P \le 0.001$) and the interaction between cropping pattern × fertilization source ($P \le 0.01$) on the phosphorus concentration of soybean were significant (Table 2). In sole cropped soybeans, soybean: black cumin (2:1) and soybean: black cumin (1:2), the addition of chemical fertilizer, enhanced phosphorus concentration by 5, 41 and 50%, respectively compared to organic manure; although, this increase was not considerable for soybean: black cumin (2:1) (Fig. 4). In contrast, in pattern of soybean: black cumin (1:1), the application of organic manure increased yield of soybean by 14%, although this increase was not significant.

Phosphorus is the least available nutrient in most soils (Raghothama, 1999). Non legume-legume intercropping can enhance rhizosphere acidification through H^+ released by roots of N_2-fixing legumes (Li et al., 2008). This rhizosphere acidification could increase dissolution of insoluble phosphorus in high pH soils (Hinsinger et al., 2003; Devau et al., 2011), and ultimately enhance soil phosphorus availability. Organic manures are a good source of phosphorus (Alizadeh et al., 2012) and can enhance phosphorus availability (Toor, 2009). A greater phosphorus enhancement through organic manure application may be attributed to several mechanisms as following: (i) insoluble Fe and Al phosphates of the soils could be converted to soluble forms by the action of organic acids such as malate, citrate, malonate, tartrate and oxalate that are released during decomposition of the organic manure (Jones et al., 2003; Westerman et al., 1988); (ii) the humate and organic acids released by organic manure may compete with phosphate ions in sorption sites and as a result prevent the phosphate fixation (Arai et al., 2005; Nagarajah et al., 1970); (iii) The enhancement of the solubility of Ca and Mg phosphates may occur due

Table 2
Analysis of variance (P value) for effect of cropping system on phosphorus concentration of soybean and black cumin and essential oil yield of black cumin under contrasting fertilization.

Source of variation	Phosphorus concentration		Essential oil yield
	Soybean	Black cumin	Black cumin
Replication	0.05	NS	NS
Cropping system (C)	0.001	NS	0.001
Fertilization source (F) (dS/m)	0.001	NS	0.001
C × F	0.01	NS	NS

NS represents non-significant.

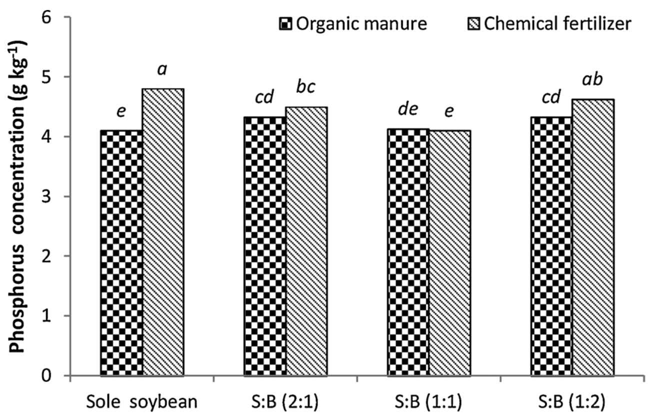

Fig. 4. The effect of cropping system on phosphorous concentration of soybean under contrasting fertilization. Means with different letters are significantly different, according to LSD test at $P < 0.05$. S and B are presented soybean and black cumin, respectively.

to carbon dioxide production during organic matter decay (Dao and Cavigelli, 2003); and (iv) humate may form a protective surface over colloidal sesquioxides and consequently decrease phosphorus fixation (Ryan et al., 2001).

Hence, phosphorus content in soybean seed is reduced in some intercropping patterns with the application of organic manure (Fig. 4). The decrease of phosphorus concentration in soybean seed can be possibly attributed to phosphorus allocation to the nodules of root. Generally, up to 20% of the plant phosphorus can be allocated to the roots' nodules of N_2-fixing legumes (Sulieman and Tran, 2015). Several evidences support the phenomenon that N_2 fixation of the plants demands more phosphorus for optimal functioning compared to other plants. The high phosphorus requirement may be related to crucial role of this nutrient in nodule energetic transformations (Sulieman and Schulze, 2010). Nodule metabolism requires high amounts of phosphorus, mainly in the form of ATP (Adenosine-5′-triphosphate) during N_2 reduction (Xavier and Germida, 2002).

The ANOVA analysis showed the phosphorus concentration of black cumin were not affected by cropping plant, fertilization source and their interactions (Table 2).

3.3. LER of the intercropping system

The land equivalent ratio value in the pattern of soybean: black cumin (2:1) treated with chemical fertilizer, was greater than 1 (Table 3). The partial LER of soybean and black cumin (based on the seed yield at maturity stage) decreased along with reducing the proportion of each crop in both fertilization sources. The lowest and highest LER $_{\text{black cumin}}$ was obtained in soybean: black cumin (2:1)

Table 3
Land equivalent ratio (LER) values of soybean and black cumin in different intercropping systems under contrasting fertilization.

Treatments	$LER_{soybean}$	$LER_{black\ cumin}$	LER_{total}
Soybean: Black cumin (1:2) × Organic manure	0.36	0.62	0.99
Soybean: Black cumin (1:2) × Chemical fertilizer	0.24	0.42	0.67
Soybean: Black cumin (1: 1) × Organic manure	0.50	0.36	0.86
Soybean: Black cumin (1:1) ×Chemical fertilizer	0.37	0.32	0.69
Soybean: Black cumin (2:1) × Organic manure	0.67	0.27	0.94
Soybean: Black cumin (2:1) × Chemical fertilizer	0.79	0.26	1.06

under chemical fertilizer and soybean: black cumin (1:2) treated with organic manure, equal with 0.26 and 0.62, respectively. Additionally, the lowest and highest $LER_{soybean}$ were achieved in soybean: black cumin (1:2) and soybean: black cumin (2:1) received chemical fertilizer, equal with 0.24 and 0.79, respectively.

Except the soybean: black cumin (2:1) which received chemical fertilizer, LER values were lower than one, that indicates no advantage of intercropping system compared with the sole crops. The land equivalent ratio value in the patterns of soybean: black cumin (2:1) received chemical fertilizer, was greater than one, indicating the advantage of intercropping. It seems that soybean had higher aggressiveness compared to black cumin.

The dominant nature of soybean may also due to soybean better ability to be the soil resource and to capture the light or a combination of both. Soybean with bigger or wider leaves has better ability to capture the light than the black cumin. Also, the height of soybean is greater than the black cumin which leads to shading effect. Moreover, in present study, soybean proportion increase in intercropping improved its growth and competitive ability and also enhanced partial LER of the soybean and total LER (Dabbagh Mohammadi Nassab et al., 2011). Fallah et al. (2018) reported in their research that in intercropping soybean with dragonhead, LER of some intercropping patterns was higher than one. Amani Amani Machiani et al., (2018a) similarly found that LER values vary from 1.06 to 1.60 in intercropping soybean with peppermint.

3.4. Yield and chemical composition of black cumin essential oil

The result indicated that the essential oil yield of the black cumin was affected by cropping pattern ($P \leq 0.001$) and fertilization source ($P \leq 0.001$); but, this trait was not affected by the interaction between cropping patterns and fertilization source (Table 2).

In the sole crop and intercropping pattern of soybean: black cumin (2:1), the essential oil yield was significantly enhanced in comparison with other intercropping patterns (soybean: black cumin (1:1) and (1:2)) (Fig. 5). The maximum essential oil yield (on an average by $0.78\ g\ m^{-2}$) was obtained in sole cropped plots. It must be noted that essential oil (E.O) is largely being considered as the products of secondary metabolism and its production is mainly related to higher levels of photosynthetic activity (Rowan, 2011). Increased yield (Fig. 2) because of increased availability of nutrients, and in particular the nitrogen (Fig. 3) and phosphorus, would increase the level of photosynthetic activity, which results in higher essential oil production in medicinal plants (Fallah et al., 2018).

The findings represented that the addition of manure could enhance the yield of black cumin essential oil by 0.94% in comparison with using chemical fertilizer. The application of organic manure leads to an increase in grow and more absorption of light (data not presented) in black cumin. Light is essential for the growth and development of medicinal plants; since, it is directly related to photosynthesis and other morphological, physiological and biochemical processes (Paez et al., 2000). The significant increase of essential oil yield parallel to increased light is directly related to the increased yield under these conditions (Silva et al., 2006).

The results of the current study are in agreement with those of Fallah et al. (2018), who underlined that the application of broiler litter enhances the yield of dragonhead essential oil by 18.40% compared to commercial fertilizer. Bajeli et al. (2016) also observed that the application of organic manure increases the essential oil yield of Japanese mint. Singh et al. (2014) discovered that the application of farmyard manure increases the essential oil yield of basil by 57% and 15.75% compared to the control (without fertilizer) and chemical fertilizer, respectively.

Thirty compounds of the total oils were identified by GC–MS analysis as shown in Table 4, representing between 98.81–99.94% of different treatments. P-cymene (20.51–62.77%), carvacrol

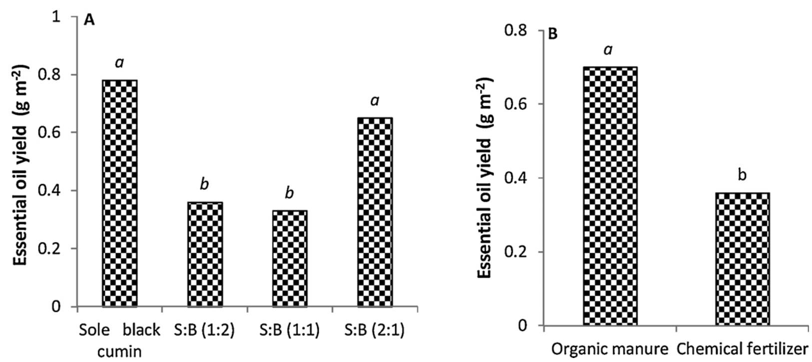

Fig. 5. The effect of cropping system (A) and contrasting fertilization (B) on essential oil yield of black cumin. Means with different letters are significantly different, according to LSD test at $P < 0.05$. S and B are presented soybean and black cumin, respectively.

(2.40–25.99%), longifolene (1.06–24.69%) and spathulenol (0.9–14.45%) were major chemical compositions of black cumin (Table 4). Harzallah et al. (2011) reported that the major components of black cumin were p-cymene, α-thujene and α-pinene. While Sunita and Meenakshi, (2013) described that p-cymene, α-thujene and dihydrocarveol were the main chemical compositions of black cumin. The quality and chemical compound of medicinal plants essential oil is determined by different factors such as nutrient management and cropping system (Weisany et al., 2016; Fallah et al., 2018).

The highest content of p-cymene and carvacrol of black cumin essential oil, were recorded soybean: black cumin (1:2) under chemical fertilizer and soybean: black cumin (1:1) with the application of

Table 4
Effect of cropping pattern on chemical compositions of essential oil of black cumin under contrasting fertilization.

			Sole black cumin		Intercropping					
			(S:B,0:1)		(S:B,1:2)		(S:B,1:1)		(S:B,2:1)	
Compounds	RI_a	RI_b	OM	CF	OM	CF	OM	CF	OM	CF
α-Thujene	926	930	1.50	5.63	2.30	12.54	6.51	1.35	4.45	3.57
α-Pinene	933	939	0.29	1.05	–	2.39	1.39	0.40	0.79	–
Sabinene	973	975	0.53	0.87	–	1.54	0.92	0.34	0.68	–
β-Pinene	978	979	0.94	1.79	–	3.13	1.93	0.72	1.10	1.17
α-Terpinene	1017	1017	0.51	0.57	–	0.86	0.27	0.23	–	–
p-Cymene	1027	1025	42.56	54.13	22.12	**62.77**	41.69	**20.51**	46	29.40
Limonene	1029	1029	–	–	–	–	–	0.11	–	–
1,8-Cineole	1031	1031	–	–	–	–	–	0.02	–	–
γ-Terpinene	1058	1060	–	–	–	0.27	–	0.56	–	–
trans-Sabinenehydrate	1097	1098	1.58	1.27	0.8	0.92	1.16	0.96	1.04	–
2-(E)-heptenyl acetate*	1120	1114	12.35	10.49	5.90	6.09	9.29	8.77	9.85	6.97
Menthol	1175	1172	–	0.54	–	0.18	–	0.25	–	–
4-Terpineol	1179	1177	2.41	1.24	0.65	0.28	1.24	1.77	1.23	–
β-Cyclocitral**	1204	–	2.29	1.98	0.97	0.88	1.21	1.50	1.67	1.75
Carvone	1248	1243	–	–	–	–	0.39	–	–	–
Thymoquinone	1254	1252	13.41	8.46	4.5	3.95	8.04	6.07	10.92	6.16
Bornyl acetate	1286	1289	0.62	0.26	–	–	–	0.61	–	–
Thymol	1302	1290	0.06	0.24	–	–	–	0.85	–	–
Carvacrol										
Carvacrol	1313	1299	8.56	7.94	12.99	**2.40**	13	**25.99**	12.65	8.11
α-Longipinene	1353	1353	1.22	0.50	–	0.28	0.70	2.78	0.61	–
			8.25							
Unknown	1380	–	–	–	–	–	1.06	–	–	–
Longifolene	1410	1408	8.25	1.87	**24.69**	**1.11**	2.89	9.32	3.50	17.59
β-Caryophyllene	1419	1419	0.45	–	–	–	–	–	–	4.99
Germacrene D	1482	1485	0.44	–	–	–	–	–	–	–
2-Tridecanone	1495	1496	–	–	1.57	–	0.40	1.16	0.32	–
Compounds	RI_a	RI_b	OM	CF	OM	CF	OM	CF	OM	CF
Citronellyl butyrate	1526	1532	–	–	9	–	0.19	0.48	0.17	–
Spathulenol	1582	1578	1.93	–	**14.45**	–	1.25	2.08	**0.90**	11.99
Caryophyllene oxide	1587	1583	–	–	–	–	0.25	0.90	0.15	–
Globulol	1589	1585	–	–	–	–	0.13	0.43	0.06	–
(E)-9-Tetradecenal**	1592	–	–	–	–	–	0.61	0.96	0.51	–
Viridiflorol	1597	1592	–	–	–	–	0.91	3.20	0.50	–
Tetradecanal	1611	1613	–	–	–	–	0.35	0.92	0.31	–
Longiborneol acetate	1684	1686	–	–	–	–	1.68	1.46	0.31	3.38
2E,6E-Farnesol	1742	1725	–	1.08	–	0.32	2.41	5.14	2.17	4.86
Total			99.9	99.91	99.94	99.9	98.81	99.84	99.89	99.94

RIa: Linear retention indices on DB-5MS column, experimentally determined using homologue series of n-alkanes.
Rib: Relative retention indices taken from Adams. *: adapted from Harzallah et al., (2011). **: adapted from NIST.
S: soybean B: black cumin OM: organic manure CF: chemical fertilizer.

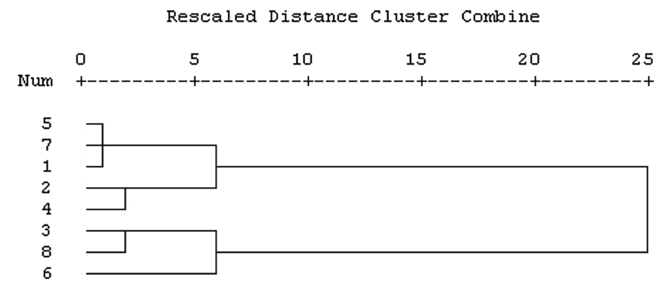

Fig. 6. Dendrogram of eight treatments of black cumin based on essential oil chemical composition using Ward clustering method. 1, 3, 5 and 7: sole crop of black cumin, soybean:black cumin (1:2), (1:1) and (2:1) with application organic manure. 2, 4, 6 and 8: sole crop of black cumin, soybean:black cumin (1:2), (1:1) and (2:1) with application chemical fertilizer.

chemical fertilizer, respectively. The highest longifolene and spathulenol content was observed in soybean: black cumin (1:2) treated with organic manure. In order to evaluate the similarities among essential oil chemical compositions of the black cumin in different cropping patterns and fertilization sources, hierarchical cluster analysis were performed (Tohidi et al., 2017). The hierarchical cluster analysis results are presented in the form of a dendrogram in Fig. 6. Chemical compositions cluster analysis of black cumin was implemented and there after essential oils were classified into two groups. The first group comprised of these components: sole cropping of black cumin treated with chemical fertilizer and organic manure, soybean: black cumin (1:2) treated with chemical fertilizer, soybean: black cumin (1:1) treated with organic manure and soybean: black cumin (2:1) treated with organic manure. These treatments were rich in p-cymene (41.69–62.77%) and carvacrol (2.4–12.65%). The second group comprised of soybean: black cumin (1:2) with the application of organic manure and soybean: black cumin (1:1) treated with chemical fertilizer and soybean: black cumin (2:1) under chemical fertilizer. They recorded high amounts of p-cymene (20.51–29.4%) and carvacrol (8.11–25.99%).

The result of our study showed that cropping pattern and fertilization sources can influence chemical compositions of essential oil of black cumin. Intercropping of soybean and black cumin enhanced the content of major chemical compositions (p-cymene, carvacrol and longifolene). A change in chemical compositions of essential oil in response to cropping pattern was observed by Weisany et al. (2016). They reported that intercropping common bean with dill enhanced carvone content. The peppermint-soybean intercropping enhanced the menthol, 1,8-cineole, neo-iso-menthol, p-menth-1-en-9-ol, (E)-caryophyllene, (E)-β-farnesene and germacrene D content and reduced the menthofuran content of peppermint (Amani Machiani et al., 2018a).

Nitrogen, phosphorus and other macronutrients are presumed as the key factors of crop growth, productivity (Bernstein et al., 2005; Alizadeh et al., 2010) and production of primary and secondary metabolites (Koeduka et al., 2006; Sharafzadeh et al., 2011; Omer et al., 2014). Although numerous studies indicated positive effect of organic manure on chemical compositions of medicinal plants (Bajeli et al., 2016; Pandey et al., 2016; Singh et al., 2014), some studies showed effect of chemical fertilizer on chemical compositions of medicinal plants (Fallah et al., 2018; Pandey et al., 2016). Nurzynska-Wierdak et al. (2013) found that fertilizers can significantly modify chemical composition of essential oils of medicinal plants. After the application of fertilizer (in the form of chemical and organic), a change in chemical composition of essential oil in medicinal plants could occur that is linked to nutritional status of the plants (Table 1). These variations are most likely associated with the changes in the synthesis pathways and the role of these components in plant physiology.

Bajeli et al. (2016) reported that the application of organic manure increases the menthol content of mint essential oil. Furthermore, Pandey et al. (2016) observed that the addition of organic manure (farmyard manure and poultry manure) increases the methyl chavicol content of basil essential oil. Singh et al., (2014) found that the application of vermicompost enhances methyl chavicol of basil essential oil compared to the control (without fertilizer) and chemical fertilizer. While Fallah et al. (2018) reported that the application of chemical fertilizer enhances the content of major chemical composition (neral, geranial and gerayl acetate) of dragonhead. Pandey et al. (2016) revealed that the addition of chemical fertilizer can enhance the linalool content of basil compared to the poultry manure.

4. Conclusion

The results of the present study showed that the yield of soybean, productivity and quality of black cumin were aff ;ected by cropping systems and fertilization sources. The highest seed yield (on an average by 247 g m^{-2}) of soybean and land equivalent ratio (1.06) were obtained in two rows of soybean plus one row of black cumin under the application of chemical fertilizer. The maximum seed yield (on an average by 86 g m^{-2}) of black cumin was obtained in sole crop of black cumin under the addition of organic manure. The p-cymene (20.51%–62.77%), carvacrol (2.40–25.99%), longifolene (1.11–24.69%) and spathulenol (0.9–14.45%) were major chemical compositions of black cumin plant. The maximum content of p-cymene and carvacrol of black cumin essential oil was obtained in one row of soybean plus two rows of black cumin under chemical fertilizer and one row of soybean plus one row of black cumin with the application of chemical fertilizer, respectively. The highest content of thymoquinone was achieved in sole cropped plants fertilized with organic manure. The highest longifolene and spathulenol content were observed in one row of soybean plus two rows of black cumin treated with organic manure. These major chemical compositions are useful for industrial use (food and pharmaceutical). According to different subjects of applying in industries it could be suggested especial treatment with favorite major compounds.

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